Automatic sample mounting and alignment system for macromolecular crystallography at the Advanced Light Source

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ABSTRACT

In an effort to realize high-throughput data collection for macromolecular crystallography, we have developed and installed an automatic cryogenic sample alignment and mounting system on the protein crystallography beam lines at the Advanced Light Source. Rapid mounting and unmounting of the samples increases the efficiency of the crystal screening process, where many crystals have to be tested for the quality of diffraction as rapidly as possible. The automounter has random access to 112 samples, stored in liquid nitrogen. Mounting of a crystal takes approximately 10 seconds, during which the crystal temperature is maintained below 120 K. Centering of a crystal can be done by the user through the remote controlled xyz goniometer head or automatically by a centering algorithm. To further increase throughput, we have also developed a sample transport/storage system based on "puck-shaped" cassettes, which can hold 16 samples each. Seven cassettes fit into a standard dry shipping Dewar.

INTRODUCTION

In the process of realizing high-throughput macromolecular crystallography, many steps of the structure determination process are being automated, from target selection to structure interpretation. These new developments are benefiting especially the structural genomics efforts and rational drug design, where large numbers of structures have to be solved.

In the present paper we are describing an automated cryogenic crystal mounting/alignment system (automounter), which is installed on the protein crystallography beamlines at the Advanced Light Source. There are several reasons for using an automounter system:

(i) Synchrotron beam time is limited and thus should be used as efficiently as possible.

(ii) Crystallography experiments are installed in radiation shielding hutches, which are inaccessible for humans during data collection. A manual sample change requires opening and closing the hutch, initiating the interlocks and performing a hutch search. This can take several minutes.

(iii) A fully automated beam line with an automounter system can be used for remote data collection, i.e. the user doesn't have to travel to the site of the synchrotron.

(iv) A fully automated beam line with an automounter system can be integrated into a higher level control system, where automated data processing and structure solving software can influence the data collection process (e.g. determining the strategy).

(v) Due to the high efficiency of the system the experimenter is enabled to select the best crystal from a large pool of samples and thus can collect better quality data.

Table 1: Comparison of manual and automated crystal mounting and centering times. The times for the manual process are rough estimates and may vary largely.

Task	Manual	Automated
Load Dewar[1]		$\approx 5\,\mathrm{s}$
Mount crystal	$\approx 5\,\mathrm{min}$	$\approx 10\,\mathrm{s}$
Center crystal	$\approx 5\,\mathrm{min}$	$\approx 30\,\mathrm{s}$

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[1] Loading or unloading the automounter Dewar with 112 samples (7 pucks) takes approx. 10 minutes, i.e. $\approx 5\,\mathrm{s/sample}$. This includes the hutch access and search.

Screen crystal (4x 30s frames)	2 min	2 min
Unload crystal	≈ 5 min	≈ 10 s
Unload Dewar[1]		≈ 5 s
Total	≈ 17 min	≈ 3 min

The significance of the automation of the crystal mounting and alignment steps in the structure determination process can be seen from Table 1. During screening of crystals (e.g. taking 4 frames), the time gain is a factor of 5-6. This doesn't take into account, that no one person can keep up the pace outlined in the table for many hours on end, whereas the automounter can screen through all 112 samples in the Dewar without human intervention.

Our design of the automounter system was driven by the following considerations. The system was supposed to be simple, easy to maintain, highly reliable, ideally suited for the given task, have a small footprint (since space at beam lines is limited) and have random access to a large number of samples stored in liquid nitrogen. At the same time technical problems related to the low temperature of the samples had to be overcome, such as: keeping the sample at LN2 temperature during the whole mounting process, icing and freezing of parts had to be avoided, thermal expansion and conductance issues had to be countered. The ALS automounting system was custom built by the LBNL Bioinstrumentation group. It is based to a large extent on pneumatic actuators, which are cost efficient, very reliable, high precision and require a simple control system.

An overview of the beamline endstation with automounter system can be seen in Figure 1. The main components of the setup are: a goniometer with a motorized goniometer head, a CCD area detector (not shown in the picture), a retractable collimator/beamstop assembly, a retractable cryostream and a long range microscope. The central element of the crystal mounting robot is the sample gripper, which is mounted on a pneumatic X-Y-Θ stage. The samples are stored in a LN2 Dewar, which is mounted on an R-Θ motorized stage.

BEAMLINE AND ENDSTATION

The first automounter was installed on beam line 5.0.3 of the Advanced Light Source, whereas a second one is being commissioned on beam line 5.0.2. A 37-pole, 16 cm period length wiggler provides hard x-rays for 3 protein crystallography beam lines, a MAD station with a Si(111) double crystal monochromator (5.0.2), and two side stations (5.0.1 and 5.0.3) with a fixed wavelength of 1.0 Å. Each side station utilizes a cylindrically bent, asymmetrically cut, horizontally focusing Si(220) monochromator crystal. The side stations are equipped with ADSC Quantum 4 detectors, the MAD beamline

with a Quantum 210 detector. The detector distance form the sample can be varied between 75-1075 mm, whereas the 2Θ angle can be as high as 46° (5.0.2 has no 2Θ capability).

Each of the three beam lines is equipped with a single-axis goniometer stage which consists of an airbearing, a servo motor and a high precision encoder. This system provides a highly variable rotation speed (0.01-360°/s), high angular resolution (1/20000°) and a small circle of confusion (<1µm at sample). A motorized XYZ or kinematic stage is mounted on the airbearing stage, which allows remote-controlled alignment of the sample (see Figure 2). The sample is viewed by a Questar SZ M100 long distance microscope (object distance up to 35.5 cm), which has variable zoom settings and can be controlled remotely (cf. Figure 1). The sample can be illuminated from the front or from the back. The Helium tank (under the microscope) contains the fast shutter and serves as an ionization chamber for beam intensity (I_0) and position measurements.

The beam collimator assembly, the beam stop, the front and backlights are all mounted on a pneumatic stage. By retracting this stage, the gripper of the automounter has clearance to access the sample. For the same reason, the cryostream is also mounted on a pneumatic stage. Additionally, both the collimator assembly and the beamstop can be remotely aligned using picomotor stages (cf. Figure 2). Currently, the exchange between three different collimator sizes (50µm, 100µm, 180µm) is done manually.

SAMPLE MOUNTING ROBOT

Figure 3 shows the sample gripper. Inside the gripper a conically shaped, brass split collet can be opened and closed by moving it in and out of a fixed tube using a small pneumatic actuator. During cool down of the gripper, a small vacuum pump is sucking LN2 into the gripper to speed up the procedure. A sensor inside the collet is used to monitor its temperature. The inner tube is surrounded by a low thermal capacity outer shroud (very thin stainless steel tube), which provides a sheath-flow of warm dry gas to reduce icing and frost formation during exposure to ambient, moisture-laden air. The upper part of the gripper, containing the pneumatic actuator and all the connections, is equipped with a heater, to keep it warm even when the lower part of the gripper is immersed in liquid nitrogen. During continuous use of the automounter the gripper is periodically warmed up to deice by an extendable heater (cf. Figure 1).

The sample gripper is mounted on a pneumatic X-Y-Θ stage. A vertical stage moves the gripper in and out of the Dewar, a 90° rotational stage can position the gripper horizontally or vertically and a long horizontal stage moves the gripper close to the goniometer head. Additionally, the gripper is mounted on a small, low-force linear stage ('small move', cf. Figure 3). This stage is used for the actual positioning of the gripper on the sample. This low-force actuator provides a

more gentle handling of the crystals and also serves as equipment protection in case of a collision.

All the pneumatic stages are equipped with magnetic Rheed-sensors, which indicate if the stage is in an extended or retracted position. These sensors not only give feedback about the state of the whole system, but they are also used to prevent collisions as part of an interlock system. Depending on the state of the automounter, certain motions are forbidden, such as the horizontal stage cannot be extended if the collimator is up or the gripper is in the Dewar. The motorized stages of the Dewar are of course also part of the interlock system.

Originally, our sample gripper was designed around the standard Hampton metal cap, which is held on the goniometer by a magnet. To improve reliability of the automounter, we are using a modified cap design, which has tight tolerances and a conical shape rather than a ledge.

Figure 4 shows the automounter Dewar, which can hold a total of 112 samples in liquid nitrogen. The Dewar is mounted on an R-Θ motorized stage, which is used to move the selected sample exactly beneath the gripper. When the gripper is being cooled down, the Dewar is moved into a position (the so called 'dock' position), so that no sample is under the warm gripper. The Dewar is automatically filled with LN2 directly from the hutch cryo system. During normal operations a cover is preventing excessive nitrogen evaporation from the Dewar. The cover can be lifted with a pneumatic actuator. For loading and unloading, the Dewar can be easily removed from the stage, by releasing one thumbscrew. Different Dewars can be placed on the stage without any realignment of components, which can further speed up the data collection process.

CONTROL SYSTEM AND DATABASE

Due to the pneumatic actuators, the automounter control system is rather simple. Digital output modules are controlling the compressed air valves, which move the stages. The sensors are connected to digital input modules. For the digital I/O functionality we are using a decentralized, modular fieldbus system form the company WAGO, which connects directly to the local area network (LAN) through Ethernet. Similarly, our GALIL XXX stepper motor controller (for the LN2 Dewar R-Θ stage) is connected to the LAN. The WAGO system also provides inputs for temperature sensors and has other functionality.

The different motions of the automounter can be actuated from a software interface either one by one by pushing 'buttons' or through scripts which run a series of motions (so called protocols). As an example, Figure 5 shows a protocol for mounting a crystal. It is started after the crystal position in the Dewar has been selected (cf. Figure 6). In step 1 the goniometer head is moved back to its standard 'zero' position, which means that the goniometer shaft is aligned with the center of rotation, to which the automounter gripper is aligned. Also the

goniometer is rotated to the angle zero. This ensures that during repeated mounting of the same crystal it is put back in the same orientation. In steps 2 and 3 the collimator and cryostream are moved out of the way, so the gripper can mount the crystal. Next, the gripper is cooled down, for which the Dewar moves to the dock position (step 4, see explanation above), the gripper is immersed (5, 6), the vacuum is switched on (7) until the temperature falls below –140°C (8,9) and the Dewar is moved back to the crystal position. Then the gripper picks up the sample (12-15), mounts it on the goniometer (17-21) and retracts (22-25). Afterwards collimator and backlight are raised (26,27), so the crystal centering can begin. Before the gripper is put back into the Dewar (32) it is warmed up for 30 s to deice it (28-31). During this time the centering and also data collection can take place.

The user interface for crystal centering is shown in Figure 6. The image of the crystal is shown in a window. In case of 'manual' crystal centering, the user clicks on the crystal and it is moved into the center by the motorized stage on the goniometer (i.e. it will coincide with the green cross on the image). Afterwards the crystal is rotated 90° and centered again. For autocentering a protocol is run, which subsequently centers the loop and rotates it by 90°. The accuracy of the autocentering algorithm still has to be improved. From the same interface the goniometer can be moved to any angle, the zoom level on the microscope can be changed and the intensity if the back- and frontlight can be adjusted.

For crystal tracking we are using a MySQL database. Here information about the crystals, such as the puck number and position where it is located, the organization it belongs to, etc. is stored. The sample cassettes are labeled with barcodes (see Figure 7), and they are scanned before they are put into the automounter Dewar. In the near future, direct connectivity to user databases is planned.

SAMPLE TRANSPORT AND STORAGE SYSTEM

To efficiently and safely transport and store frozen samples, and to be able to easily load many crystals into the automounter Dewar, we have developed a sample cassette system (cf. Figure 7). The main considerations for the design were high sample density and compatibility with the dry shipping Dewar CP100 from Taylor-Wharton.

One assembly can hold 16 samples and consists of a sample cassette (the 'puck') and a magnetic base. For transportation and storage, the cassette and the base are locked together by two springs on the side of the base. A central post in the base is used to guide the base into or out from the cassette, making sure that the crystals mounted in the cryoloops cannot hit the surface of the cassette. The noncircular shape of the post provides the correct orientation between the two parts. The magnetic sheet on the bottom of the base holds the samples in the base and also holds the base in the Dewar of the automounter (cf. Figure 7). The pucks

are labeled with cryocompatible barcodes for tracking. The tools to handle the sample cassettes and the magnetic bases are shown in Figure 8.

Seven cassette assemblies can be stored in a CP100 Dewar (cf. Figure 9). The pucks are placed between the shelves of a cylindrical holder and springs hold them in place. The samples are stored upside down, so some liquid nitrogen can be preserved in the openings of the cassettes. To secure the pucks during transportation, a metal rod is inserted into the holder through all the cassette assemblies.

RESULTS

There are several user groups utilizing the robot on a regular basis, in fact some of them are using the automounter exclusively to mount crystals on beamline 5.0.3. Consistent with the times listed in Table 1, screening of 16 crystals (1 cassette) with 4x 30 s frames takes on average 42-45 minutes. So far, during 9 months of user operation, a total of approximately 800 crystals were screened using the automounter, roughly 160 data sets were collected, which led to approx. 50 unique structures. The highest resolution data was 1.1A with an Rmerge =12% on a bacterial protein target. For several data sets Rmerge was <4%.

GNF: screened 30, collected 5, solved 5
LLNL: screened 162, collected 16, solved: 3
Syrrx: screened 600, collected 140, solved 40

SUMMARY AND OUTLOOK

In summary, we have built a cryogenic crystal mounting system for macromolecular crystallography, which can be used at synchrotron beam lines. The automounter was custom built for the specific task and it is relatively simple and low-cost, has a small footprint, is well integrated into the beam line control systems and it is very reliable. The first automounter, installed on beam line 5.0.3 of the Advanced Light Source storage ring, has been in operation for a year. A second system has been commissioned recently on beam line 5.0.2, and two more automounters are being built currently for beam lines 5.0.1 and 8.2.1. Ultimately, we plan to install crystal mounting robots on every protein crystallography beam line at the ALS.

Due to the high reliability of the automounter, no major changes or improvements of the hardware are planned currently. On the other hand, a lot of work remains to be done regarding software and database development, to make these systems as transparent and user friendly as possible, and also to further increase efficiency. Among others, connectivity and synchronization between the

users' databases and the beamline database is planned. Possibilities for remote beamline/automounter operations are also being explored. For this purpose, personal and equipment safety has to be ensured and the proper procedures have to be worked out. Last but not least, it is planned to use the automounter system as the first step in a fully automated structure solution process, such as the LBNL PHENIX project [2].

ACKNOWLEDGMENT

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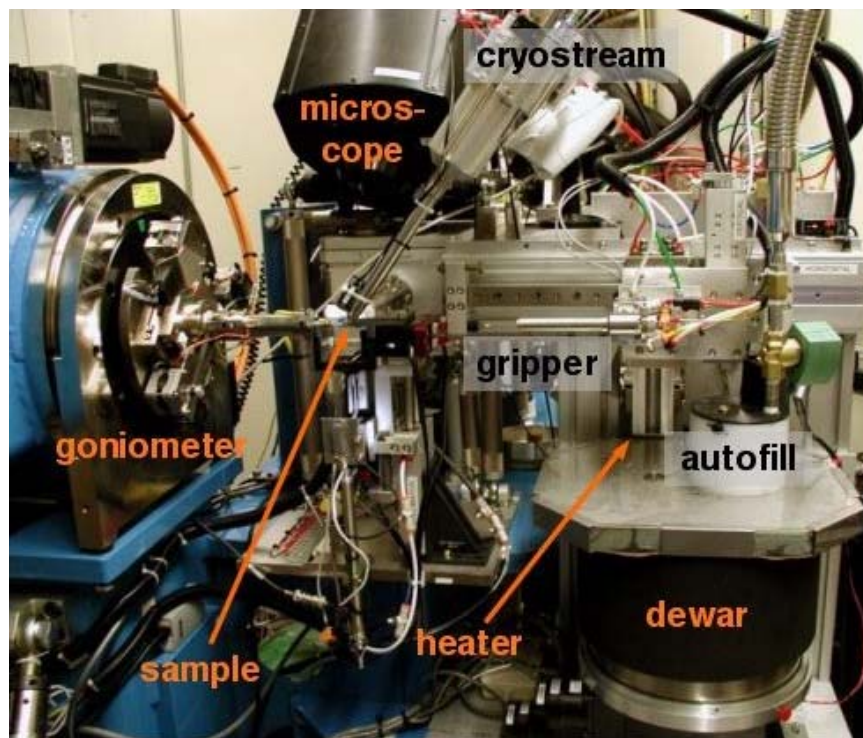
FIGURES

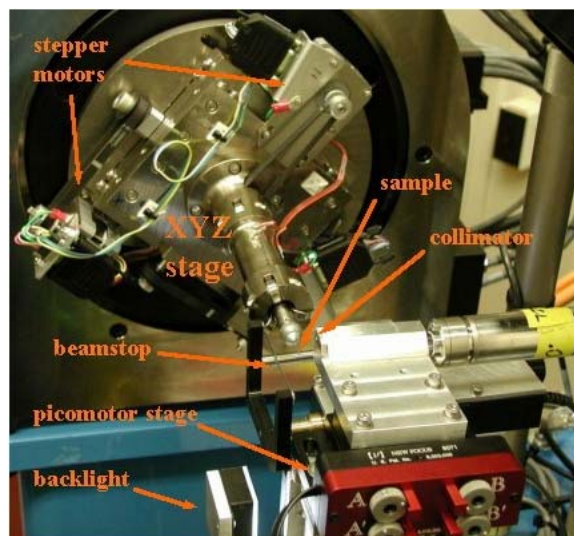**Figure 1: Overview of the ALS beamline 5.0.3 endstation with automounter.**

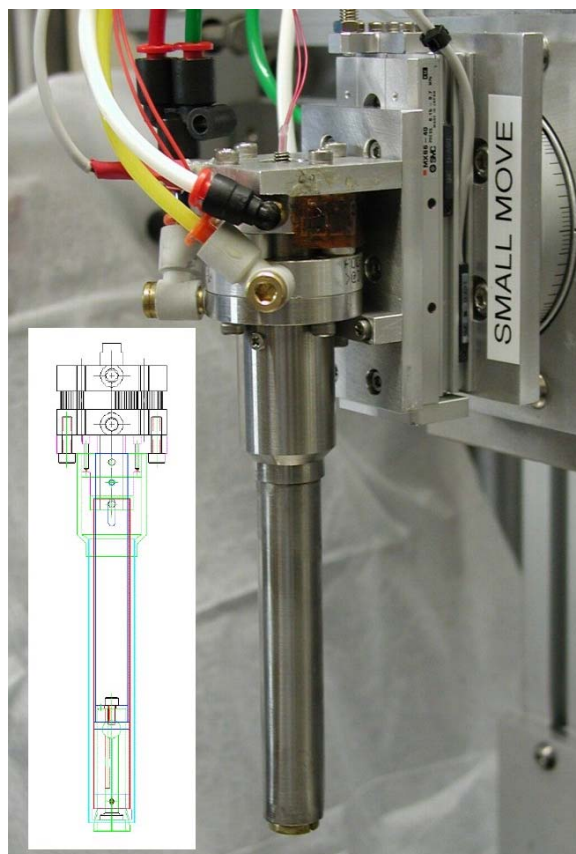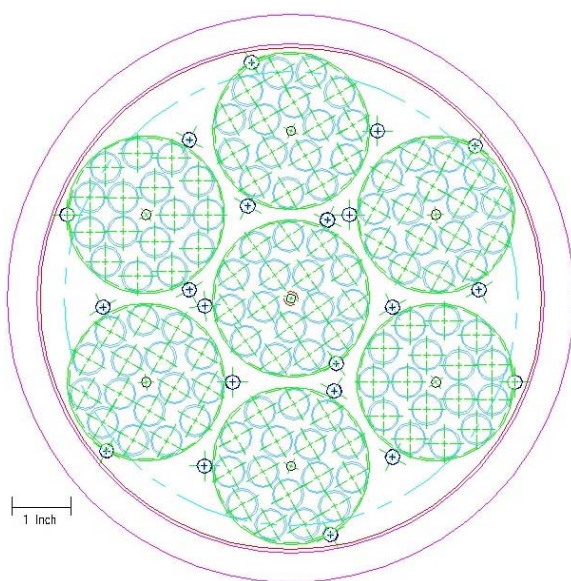**Figure 2: Goniomter, XYZ stage, collimator and beamstop assembly.**

Figure 3: Sample gripper

Figure 4: Liquid nitrogen Dewar of the automounter, which can hold a total of 112 samples (7x16).

Step	Action	Object	Amount	Wait
1	XYZ to Zero			500
2	Output	Collimator	down	10
3	Output	Cryostream	retracted	10
4	Move To	Dock		10
5	Output	UpDown	down	10
6	Out noWait	Small move	extended	100
7	Output	Vacuum	on	10
8	Temp below	Grip	-140	10
9	Output	Small move	retracted	100
10	Output	Vacuum	off	10
11	Move To	Previous		100
12	Output	Small move	extended	100
13	Output	Grip	grip	250
14	Output	Small move	retracted	10
15	Output	UpDown	up	100
16	Output	Nitrogen	on	10
17	Output	Rotate	up	100
18	Output	Horizontal	extended	10
19	Output	Small move	extended	200
20	Output	Nitrogen	off	10
21	Output	Grip	release	150
22	Output	Small move	retracted	133
23	Output	Cryostream	extended	10
24	Output	Horizontal	retracted	10
25	Output	Rotate	down	100
26	Output	Collimator	up	10
27	Output	Backlight	up	10
28	Output	Heater	extended	10
29	Output	Hot air	on	30000
30	Output	Hot air	off	10
31	Output	Heater	retracted	10
32	Output	UpDown	down	10

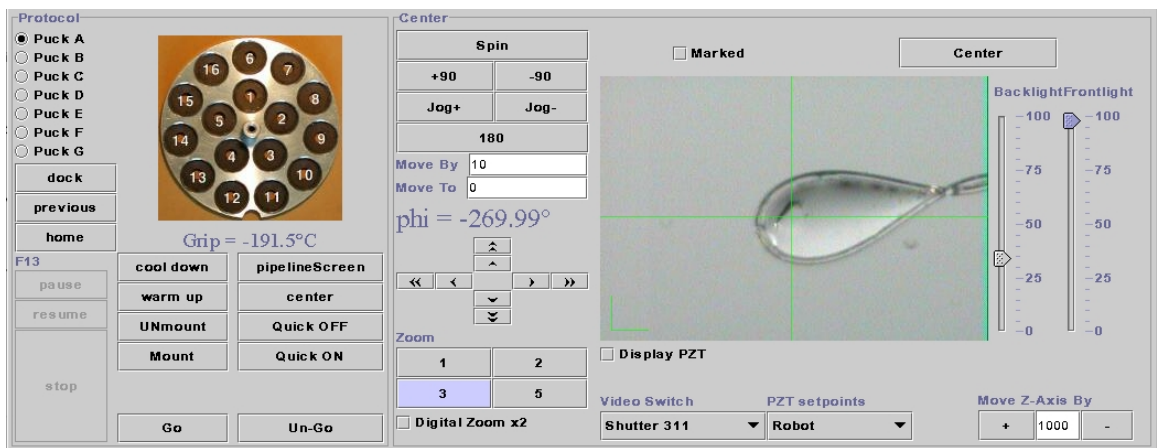**Figure 5: Protocol for mounting a sample.**

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Figure 6: Part of the graphical user interface used to select samples in the Dewar and mount/unmount them (left) and the crystal centering window (right). For details see text.

Figure 7: Sample cassettes ('pucks') and magnetic bases.

Figure 8: Tools to handle the cassettes and bases. From left to right: bent forcep, straight forcep, pusher, base holder. The forceps are used to hold the cassettes. The pusher is used to separate a cassette from the base when they are closed, the base holder is used to hold a base when it is being inserted into a cassette.
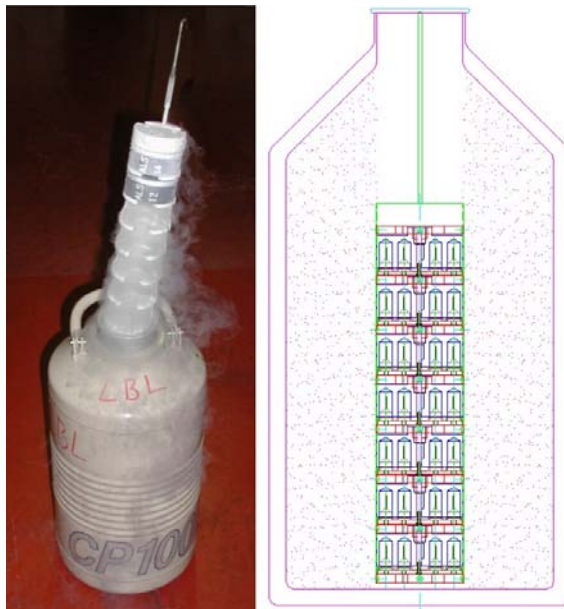

Figure 9: Left: a sample cassette holder with two cassettes is being removed from a Taylor-Wharton CP100 dry shipping Dewar. Right: cross section of the CP100 with sample holder inserted.